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RAT LIVER TYROSINE AMINOTRANSFERASE ACTIVITY AND INDUCTION BY DEXAMETHASONE UPON CADMIUM INTOXICATION

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Abstract – This study was focused on Cd effects on basal and dexamethasone–induced tyrosine aminotransferase (TAT) activity in the rat liver cytosol. Cadmium (Cd), applied in the dose of 2 *mg/kg* b.w., stimulated both TAT activity and its induction by dexamethasone, inducing the most prominent alterations 24 *h* after administration. Doses lower than 2 *mg* Cd/*kg* b.w. were ineffective, while the higher ones (3 and 4 *mg* Cd/*kg* b.w.) led to the changes similar to those reached by 2 *mg* Cd/*kg*. The *in vitro* application of different Cd concentrations to the liver cytosol rendered the enzyme activity unchanged, suggesting that the metal acted at the level of TAT gene transcription.

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INTRODUCTION

Cadmium (Cd) is a nonessential heavy metal accountable for many toxic effects in various living organisms, including humans (Goering *et al.* 1995). Although the molecular mechanisms responsible for Cd toxicity are not well understood, several lines of evidence suggest that it relies on a high affinity of the metal for protein thiol groups and/or on its competition with essential metal ions (Vally and Ulmer 1972; Stacey 1986).

It is well documented that Cd, among other heavy metals, could modify the activity of numerous enzymes in mammalian cells (Davalli *et al.* 1989; Zhang *et al.* 1990; Long 1997). So, its stimulatory effect on hepatic gluconeogenesis was shown to be based on the increased activity of some key gluconeogenic enzymes (Chapatwala *et al.* 1982). Cd–related changes in an enzyme activity might result from the metal interference with the enzyme essential thiols, with enzyme-substrate complex formation, or with the mechanisms regulating the transcription of the relevant gene. It was reported that Cd could enhance transcription of several genes, such as those coding for metallothionein, heme oxigenase, several heat shock proteins and some other proteins (Beyersmann and Hechtenberg 1997), but molecular mechanism(s) underlying these influences are still the matter of a debate.

Tyrosine aminotransferase (TAT; L-tyrosine 2-oxoglutarate aminotransferase, E.C.2.6.1.5.), the rate-limiting enzyme of tyrosine catabolic pathway, is one of the key gluconeogenic enzymes which is almost exclusively expressed in hepatic parenchymal cells. In its primary structure, TAT contains 17 cysteines, which make this enzyme a possible target for Cd ions (Seralini *et al.* 1995). Regulation of TAT gene expression is complex and under control of several hormones, glucocorticoids and glucagon being positive, and insulin being a negative regulator of its expression (Reik *et al.* 1994).

In this paper Cd effects on basal and dexamethasone-induced TAT activity in the rat liver cytosol are presented. Parallel *in vitro* and *in vivo* treatments with Cd were applied in order to learn whether Cd affects TAT gene transcription or the pre-existing enzyme activity.

MATERIAL AND METHODS

Animals and treatment

Male rats of Wistar strain (2-2.5 months old; 200-250 *g* b.w.) were reared under standard laboratory conditions with 12:12 *h* light/dark cycle, at 22°C. The animals were divided into two groups, each consisting of at least three rats. In the dose-dependent experiments $CdCl_2$ dissolved in 0.9% NaCl was administered i.p. at 0.5-4 *mg* Cd/*kg* b.w., 24 *h* before death, while in the time-dependent ones $CdCl_2$ was administered i.p. at 2 *mg* Cd/*kg* b.w. and the rats were sacrificed 2, 24 or 48 *h* after the injection. Control rats received equivalent volume of 0.9% NaCl. Dexamethasone (5 *mg/kg*) was injected i.p., 4 *h* before death.

Assay for tyrosine aminotransferase activity

The assay was done essentially as described by Diamondstone (1966). The livers were homogenised in 9 vol (w/v) of cold 0.14 M KCl and the supernatant obtained after centrifugation (45 min, 30 000 *g*) was used as a source of the enzyme. The incubation mixture, in a final volume of 3.2 mL, contained: 6 mM L-tyrosine dissolved in 0.2 M potassium phosphate buffer, pH 7.3, 9.4 mM α-ketoglutaric acid, 0.038 mM pyridoxal-5-phosphate, 3.8 mM diethyldithiocarbamic acid and 0.2 mL of the supernatant diluted 5- to 8-fold with 0.14 M KCl. The reaction was initiated by the addition of the enzyme, run for 10 min at 37° C and terminated by 0.2 mL 10 N NaOH. The absorbance at 331 nm was measured after 30 min at room temperature against the null-time probes. Enzyme activity was expressed as mmol of the reaction product, p-hydroxybenzaldehyde (pHBA), *per* min *per* milligram protein.

Miscellaneous

Total Cd content in the liver cytosols was determined by atomic absorption spectrometry (a Perkin Elmer 4000) after HNO_3-digestion of cytosols.

Protein content in the liver cytosols was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

RESULTS

In order to establish whether Cd (2 *mg* Cd/*kg* b.w) influences TAT activity and induction of the enzyme by dexamethasone (5 *mg*/*kg* b.w.), the rats were sacrificed 2, 24 or 48 *h* after the metal administration and the enzyme assay was performed in the liver cytosol. Dexamethasone, as a potent glucocorticoid agonist, was administered 4 h before the sacrifice. The results showed that 2 *h*, 24 h, and 48 *h* after the administration Cd led to the increase in TAT activity in the liver cytosol by 17%, 79% and 35% over the basal level,, respectively (Fig. 1, open bars). Besides, at the end of 24 and 48 *h* time period after Cd administration, the extent of TAT induction by dexamethasone (Fig. 1, full bars) was elevated by 101% and 45%, respectively.

The influence of different Cd doses (0.5 to 4 *mg* Cd/*kg* b.w.) on TAT activity in the liver cytosol and on its induction by dexamethasone was tested 24 *h* after the metal administration. Low Cd doses (0.5 *mg* and 1 *mg* Cd/*kg*) did not alter TAT activity, while intermediate

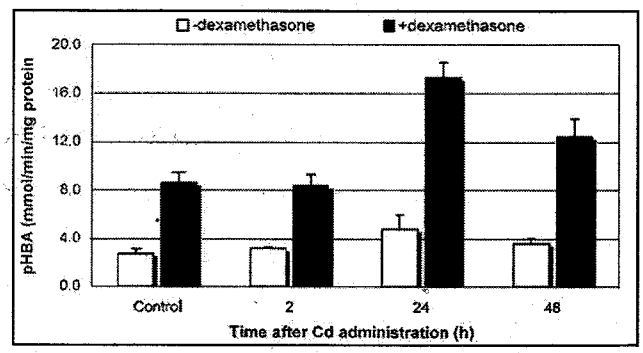

Fig. 1. TAT activity and induction by dexamethasone after different time intervals of Cd treatment. The enzyme activity was determined in liver cytosol of rats sacrificed 2 *h*, 24 *h* and 48 *h* administration of 2 *mg* Cd/*kg*. Dexamethasone was administered 4 *h* before death at 5 *mg*/*kg*. The control bars represent the enzyme activity in the cystol of rats injected with saline instead of $CdCl_2$. The values are means ± SE from three independent experiments done in triplicates

and high ones (2 *mg*, 3 *mg* and 4 *mg* Cd/*kg*) affected TAT activity, elevating it 2.2-, 3.0- and 2.4-fold over thelevel determined in the cytosol of control rats, respectively (Fig. 2, open bars). The changes in TAT induction by dexamethasone provoked by different Cd doses displayed a similar trend: low doses (0.5 *mg* and 1 *mg* Cd/*kg*) caused only a small and insignificant reduction of the enzyme induction by the glucocorticoid, while the higher ones stimulated TAT induction by dexamethasone nearly 2-fold over the hormone-induced level in the absence of the metal (Fig. 2, full bars).

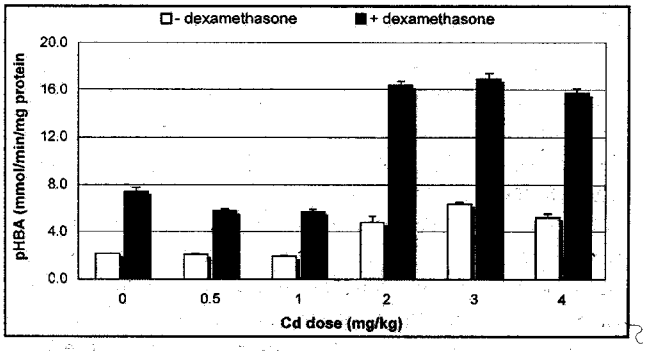

Fig. 2. TAT activity and induction by dexamethasone in rats injected with different Cd doses. The enzyme activity was determined in liver cytosol of rats sacrificed 24 *h* after administration of indicated Cd doses. Dexamethasone was administered 4 *h* before death at 5 *mg*/*kg*. The values are means ± SE from three independent experiments done in triplicates

A possibility that Cd-provoked changes in the enzyme activity are the consequence of the metal influence on TAT gene transcription was checked by comparing TAT activity in the liver cytosol after *in vivo* and *in vitro* Cd application. For the *in vitro* experiments, Cd concen-

trations ranging from 2 to 16 μg/mL were selected such as to be comparable to those determined by atomic absorption spectrometry in the liver cytosol after *in vivo* administration of different Cd doses (Table I). It was found that the metal added *in vitro* did not significantly influence the enzyme activity irrespectively of the concentration applied (Fig 3).

Table 1. Concentration of Cd in the liver cytosol of rats injected with different Cd doses

Administered Cd dose (mg/kg b.w.)	Cd concentration by AAS* (μg/mL cytosol)
Control	0.00
0.5	1.92 0.31
1	4.89 0.26
2	7.31 0.18
3	9.97 0.48
4	12.78 0.38

* The values represent the means SE from four independent determinations of Cd concentration by atomic absorption spectrometry.

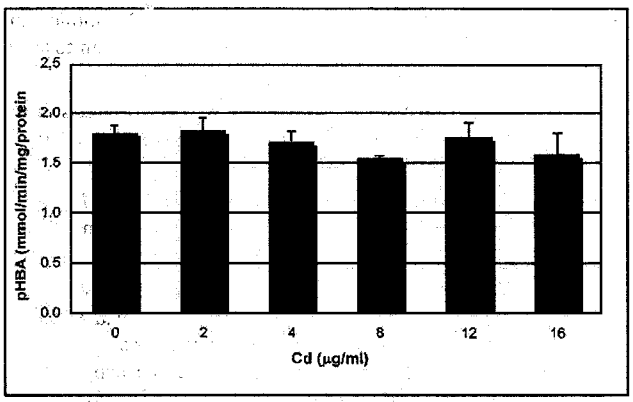

Fig. 3. In vitro effect of Cd on TAT activity. The enzyme activity was determined in liver cytosol of rats preincubated for 2 *h* at 0°C with indicated Cd concentrations. The values are means ± SE from three independent experiments done in triplicates

DISCUSSION

TAT is one of the four key gluconeogenic enzymes and its role is to convert tyrosine into *p*-hydroxyphenylpyruvate. Modification of this enzyme activity, as well as of its gene expression under different conditions influences tyrosine metabolism and availability to extra hepatic tissues. In order to check for a possibility that alteration of TAT activity may represent a part of Cd toxicity, this study was focused on Cd effects on TAT activity and on induction of the enzyme by dexamethasone. Comparison of these effects after *in vitro* and *in vivo* application of Cd enabled us to differentiate its transcriptional effects from the cytosolic ones.

Cd–induced changes of TAT activity and of its induction by dexamethasone were noticed 24 *h* after administration of the metal at doses higher than 2 *mg/kg*. The lack of Cd effects at 2 *h* time after the treatment could be explained by a too short time interval. At 48 *h* after Cd administration, only dexamethasone induction of the enzyme was affected, but the extent of the induction was lower than at 24 *h*. It could be assumed that metallothionein, induced by the metal in a meantime, took a protective role over the enzyme. Our previous results have shown that the concentration of metallothionein in the rat liver cytosol reached its maximal level 48 *h* after Cd injection (Dundjerski *et al.* 1991), and also that Cd doses of 0.5-2 *mg/kg* were more potent in metallothionein induction than the higher ones (Bauman *et al.* 1993; Dundjerski *et al.* 2000a). As a sulphydryl rich protein, metallothioneins are assumed to be the first and the most important components of Cd and other heavy metal detoxification mechanisms (Dundjerski and Matićć 1997; Klaassen *et al.* 1999; Roesijadi, 2000). They act as efficient scavengers of Cd ions *via* numerous SH groups, thus disabling the metal ions to interact with essential thiols of other proteins (Roesijadi 2000). Apart from metallothioneins, previous studies from our and other laboratories revealed that Cd was also an inducer of heat shock proteins, as key components of general protection mechanisms under stress conditions (Goering *et al.* 1993; Dundjerski *et al.* 2000a; Dundjerski *et al.* 2000b).

There are several possible mechanisms responsible for elevated TAT activity and induction of the enzyme by dexamethasone in the liver of Cd–treated rats. First, it is possible to speculate that elevated TAT activity in our experiments could be, at least in part, a consequence of Cd effects on Ca^{2+} homeostasis. This assumption is supported by the observations that ursodeoxycholic acid stimulation of TAT induction by dexamethasone in cultured rat hepatocytes is mediated *via* protein kinase C (PKC) signal transduction pathway (Mitsuyoshi *et al.* 1997), and that Cd provokes disturbance in Ca^{2+} metabolism and modulates PKC activity in different cell lines (Block *et al.* 1992; Long 1997). Second, according to a new model for transcriptional regulation of the

TAT gene expression (Simons *et al.* 1992) which includes glucocorticoid responsive *cis*-acting elements (GRE) and their cooperation with additional modulatory elements (GME), it could be assumed that the alteration of TAT activity may be a consequence of Cd interaction with one or more transcription factors responsible for the TAT gene regulation. Third, it could be supposed that Cd effects are mediated through glucocorticoid receptor. This protein acts as a hormone-inducible transcription factor involved in TAT gene regulation and its most important functional properties, such as hormone binding capacity and binding to DNA, were shown to be affected by Cd (Dundjerski *et al.* 1992). However, the fact that these changes were opposite to those expected to underlie Cd effects described herein, makes this mechanism less likely. Finally, it could be speculated that Cd could influence TAT activity by various mechanisms, possibly modulating the cross–talk between different signal transduction pathways.

The assay of TAT activity in hepatic cytosol after *in vitro* and *in vivo* Cd application revealed the influence of the metal only when applied *in vivo*, suggesting that its effects are expressed at transcriptional level. This suggestion is strengthened by the observation that Cd effects on the enzyme activity and on its induction by dexamethasone exert a parallel trend.

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АКТИВНОСТ ТИРОЗИН АМИНОТРАНСФЕРАЗЕ У ЈЕТРИ ПАЦОВА И ИНДУКЦИЈА ЕНЗИМА ДЕКСАМЕТАЗОНОМ У УСЛОВИМА ИНТОКСИКАЦИЈЕ КАДМИЈУМОМ

ЈАДРАНКА ДУЊЂЕРСКИ, ЈЕЛЕНА ПРЕДИЋ, АЛЕКСАНДРА ЧВОРО и ГОРДАНА МАТИЋ

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Истраживања су била усмерена ка праћењу ефеката Cd на основну и дексаметазоном индуковану активност тирозин аминотрансферазе (ТАТ) у цитосолу јетре пацова. Кадмијум (Cd) примењен у дози од 2 *mg/kg* телесне тежине, стимулисао је и активност ТАТ и индукцију ензима дексаметазоном. Најизраженије промене су запажене 24 *h* након ињецирања метала животињама. Дозе ниже од 2 *mg/kg* телесне тежине нису утицале на активност ензима, док су промене у присуству виших доза (3 и 4 *mg Cd/kg* телесне тежине) биле приближне оним запаженим након третирања животиња са 2 *mg* Cd/*kg*. Непромењена активност ензима уочена након инкубирања цитосола јетре пацова са различитим концентрација Cd, сугерисала је да се утицај метала остварује на нивоу транскрипције гена за тирозин аминотрансферазу.